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14. ABSTRACT

Previously we documented that T cells from normal individuals express complement on the surface membrane after stimulation. We discovered there is a significant difference in the distribution of complement proteins on T cells from SLE patients compared to other autoimmune diseases and normal individuals and that complement deposition (C4d and C3d) is associated with SLE disease activity. We also discovered that complement deposition affects T cell function and decrease (or goes along with) Ca flux, IL- 2 production and cytotoxic activity.
During this past year the following has been accomplished.
1. C4d bound to critical surface membrane proteins of SLE T cells – Lipid rafts and CD3.
2.C4d is associated with aberrant signal transduction and additional downstream effects, which in turn may contribute to T cell dysfunction and overall abnormalities of the immune system in SLE patients.
3.There is increased cytokine production by C4d + SLE T cells.
4.There is increased cytokine production by C3d+ T cells.
5.C3d is partially co-localized within aggregated lipid rafts on a subpopulation of SLE T cells.

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Introduction:

The complement system plays an important role in protection against pathogenic organisms and modulates several aspects of the humoral and cellular immune response. Complement is invariably activated in patients with infectious and autoimmune diseases including systemic lupus erythematousus (SLE). Complement activation is linked to the expression of tissue pathology through either the formation of immune complexes or its direct deposition on tissues and cell surface membranes. The complement membrane attack complex C5b-9 binds to cell membranes, independent of any receptor, and it may activate multiple signaling pathways. Complement is known to alter B and T cell function. In B cells, it lowers the activation threshold and supports the development of B-cell memory and immune tolerance. Complement modulate antigen- presenting cell (APCs) and indirectly T cell function. The complement system and its regulatory proteins have an important role in the determination of T cell responses by modulating T cell apoptosis and inducing regulatory T cells. Recent studies have shown that complement can initiate or modulate a surface membrane signaling response in T cells. However, the underlying signaling pathways involved in these effects are still obscure.

SLE and complement system. There is a strong relationship between the complement system and SLE, complement levels are decreased in patients with active SLE. Immune complexes consisting from complement and auto- antibodies are deposited in the glomeruli of patients with lupus nephritis and complement fragments are used as a biomarkers for disease activity. One would assume that deficiency in complement would protect patients from SLE. On the contrary, hereditary homozygous deficiency of

different complement proteins predispose patient to SLE. Few theories were developed, trying to explain the contradicting observations, with emphasis on the role of complement on waste-disposal of aged and damaged cells and on maintaining immune tolerance by modulating B and T cell activity. The complex interaction between T cells and the complement system is still obscure but significantly important.

Ahearn and colleagues have found that complement activation fragments C3d and C4d are deposited on the surface membrane of erythrocytes and lymphocytes in patients with SLE. The deposition of C4d on the surface of red cells may represent a clinically useful disease biomarker. These observations lead to the hypothesis that lymphocyte-bound C3d and C4d may also serve as biomarkers for lupus diagnosis. In addition, deposition of the complement fragments C4d and C3d on the surface of T cells has unknown as of yet functional repercussions. T cells are major contributors to SLE pathogenesis. SLE T cells display aberrant cell surface mediated signaling responses, and they produce increased amounts of IL-17 although the production of IL-2 is compromised. Interestingly, lipid rafts, cholesterol rich domains on the surface of cells harboring signaling molecules aggregate on the surface membrane of SLE T cells and contribute to disease pathology in lupus-prone mice. We assumed that the complement activation product C3d might alter the functional activity of these T cells.

Body:

In this report we will present the data gathered during the preceding year of the grant.

Aim 1 of the grant: descriptive studies

"To characterize the phenotype of peripheral blood T cells of SLE patients, with respect to deposition of complement activation products as well as expression of lineage-specific markers, complement receptors, complement regulatory proteins, adhesion molecules, and distribution of lipid rafts"

Localization of C4d on the surface of SLE T cells

An interesting observation that we have made was that C4d is significantly highly deposited on the surface of SLE T cells). Initial characterization of T cells from SLE patients, patients with other diseases, and healthy controls failed to detect surface-expression of complement receptor (CR) 1, CR2, and Fc receptors (FcR) (data shown in the 2009 report), suggesting that C4d is unlikely to bind through CR and FcR to the surface of SLE T cells. We speculated that, if C4d depositions are deposited at or near lipid rafts, they may gain close access to the TCR/CD3 complexes and consequently alter downstream signaling processes. To investigate the localization of C4d on the surface of SLE T cells confocal microscopy was performed. As shown in Figure 1B of our publication (Third manuscript PDF (Liu et al) attached in the final report), C4d appeared to be present on SLE cells in various patterns, ranging from a punctuate pattern with a concentrated "cap" (upper panels) to a diffuse distribution (middle panels). Interestingly, C4d appeared to co-localize with CD3 (upper panels) and lipid rafts (middle panels) on SLE T cells.

Because lipid rafts are known to be enriched in TCR/CD3 complexes and other signaling molecules, SLE T cells were also stained with CT-B (cholera toxin B which is known lipid raft marker) and anti-CD3 to ensure the authenticity of the staining patterns observed (lower panels). Together, these results suggested that C4d is associated with signaling molecules in lipid rafts of SLE T cells**.**

To further verify the possibility that C4d may covalently bind to surface molecules that are essential for T cell signaling (e.g., the TCR/CD3 complex), co-immunoprecipitation experiments were performed. As revealed by the representative results shown in Fig. 2 of Liu et al attached in the final report), an anti-C4-reative protein (M_r ~65-70 kD) was present in the eluate of anti-CD3-coated beads, but not in that of mouse IgG-coated beads. The apparent molecular mass of this molecule suggests that it is a complex of C4d (M_r 45 kD) and a subunit of the TCR/CD3 complex (e.g., CD3γ, CD3ε, or CD3δ; M_r ranging from 20-23 kD). This result implied that C4-derived products are associated with the TCR/CD3 complexes in SLE T cells and thus may play a role in perturbing T cell signaling and functioning.

Aim 2 of the grant: functional studies

"To investigate the functional consequence of deposition of complement activation products on T cells using both ex vivo and in vitro systems, including localization of complement activation products, their spatial and functional relationships with TCR, other T cell surface molecules and lipid rafts, and T cell signaling"

Abnormal signaling processes in C4d-bearing SLE T cells

The myriad abnormalities reported for SLE T cells may, at least partially, stem from dysregulated signaling processes in these cells. Therefore, it is important to determine if the intracellular signaling pathways are abnormal in SLE T cells and whether abnormal signaling processes may be associated with C4d deposition on T cells. Measurement of phosphorylation of signaling proteins has commonly been performed to investigate signaling pathway. Thus, intracellular phosphoprotein staining studies were performed to examine the Jak-Stat pathway and MAPK/ERK pathway, two important families of signaling proteins that regulate immune response, in T cells. Specific responses of these signaling pathways to different stimuli occurred in an expected manner. However, erratic responses were detected in T cells from SLE patients (Fig. 3 of Liu et al attached in the final report). The signaling responses to cytokines and mitogens appeared to remain relatively normal in SLE T cells bearing none or low levels of C4d. However, these signaling proteins appeared to respond hyperactively or non-specifically in T cells bearing high levels of C4d. These results not only demonstrated irregular signaling processes in SLE T cells, but also implied a causative relationship between the binding of C4d to T cell signaling molecules and aberrant signaling events.

C3d partially co-localized within aggregated lipid rafts on a subpopulation of SLE T cells.

To investigate this possibility that C3d deposit into the lipid rafts, we used wide-filed fluorescence microscopy, followed on some cases by de-convolution to analyze the cell surface distribution of the cholera toxin B (CTB) conjugated to AF- 488 (green) and an

anti-C3d antibody conjugated to AF- 568 (red). (fig 4, Borshukova et al attached to the final report) Even though the SLE T cells were not stimulated, in a subpopulation of T cells the lipid rafts were clustered, as reported previously. By microscopical analysis, the percentage of double positive T cells (for both C3d and CTB) represented about 10% of all the cells investigated. Taken together, these results suggest that in a subpopulation of SLE T cells, about 40% of the cell surface C3d fragments bind to lipid rafts.

Cytokine production by C3d+ T cells is increased

To assess the functional importance of C3d fragments we compared the profile of cytokine production between the C3d+ T cell population and the C3d- T cell population and between the different study groups. We used conjugated antibody against C3d combined with intracellular staining for different cytokines and recorded the data using flow cytometry studies. The production (by the whole T cell population) of IL-2 and IFN-gamma was decreased in the SLE T cells when compared with T cells from normal ($p<0.05$) and OAD patients ($P<0.05$). However, the subpopulation of C3d+ T cells produced significantly more IL-2, IFN-γ, IL-4 and IL-17 (Figure 3, Borshukova et al, attached to the final report) when compared to the C3d- T cells. The increased production of various cytokines by the C3d+ T cell population was not limited to T cells from SLE patients and a similar pattern was observed in T cells from patients with OAD and normal subjects.

Key research accomplishment:

1. C4d bound to critical surface membrane proteins of SLE T cells – Lipid rafts and CD3.
2. C4d is associated with aberrant signal transduction and additional downstream effects, which in turn may contribute to T cell dysfunction and overall abnormalities of the immune system in SLE patients.
3. There is increased cytokine production by C4d + SLE T cells.
4. There is increased cytokine production by C3d+ T cells.
5. C3d is partially co-localized within aggregated lipid rafts on a subpopulation of SLE T cells.

Reportable outcome:

Publications derived from this project are presented in the final report

Conclusion:

C4d bounds to critical surface membrane proteins of SLE T cells may lead to aberrant signal transduction and additional downstream effects, which in turn may contribute to T cell dysfunction and overall abnormalities of the immune system in SLE patients.
C3d fragments are localized in the lipid rafts of SLE T cells and contribute to abnormal T cell function by increasing cytokine production. The increased numbers of C3d+ T cells in patients with SLE may though contribute to the immunopathogenesis of the disease because increased production of IL-4, and IL17 may further advance the inflammatory response and tissue damage.

References

None

Appendices:

None

Supporting data:

None